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U.S. Environmental Protection Agency
Office of Prevention, Pesticides, and Toxic Substances
Office of Science Coordination and Policy

Scope of Work

for

Endocrine Disruptor Screening and Testing Standardization and Validation Technical Support Services

1.0 INTRODUCTION

The United States Environmental Protection Agency is implementing an Endocrine Disruptor Screening Program (EDSP). This procurement entails comprehensive toxicological and ecotoxicological testing services, including the chemical analytical, statistical and Quality Assurance/Quality Control support, to assist the Agency in developing, standardizing, and validating a suite of *in vitro*, mammaliam, and ecotoxicological screens and tests for identifying and characterizing endocrine effects in pesticides, industrial chemicals, and environmental contaminants. The studies conducted will be used to develop, standardize and validate methods, prepare appropriate documents for peer review of the methods, and develop technical guidance and test guidelines in support of Office of Prevention, Pesticides and Toxic Substances regulatory programs.

2.0 BACKGROUND

In 1996, Congress passed the the Food Quality Protection Act (FQPA) and Amendments to the Safe Drinking Water Act (SDWA) which require EPA to:

> *"develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate."*

To assist the Agency in developing a pragmatic, scientifically defensible endocrine disruptor screening and testing strategy, the Agency convened the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). Using EDSTAC (1998) recommendations as a starting point, EPA proposed an Endocrine Disruptor Screening Program (EDSP) and requested public comments on its proposal in the *Federal Register* (63 FR 42852; 63 FR 71542). In April 1999,

EPA convened a formal scientific peer review of the EDSP proposed statement of policy (63 FR 71542) by a joint committee of the U.S. EPA Science Advisory Board and the FIFRA Scientific Advisory Panel (SAP/SAB 1999).

3.0 SCOPE OF WORK

3.1 General Considerations

The scope of this effort includes the conduct of laboratory studies and associated analyses to validate the assays proposed for inclusion in EPA's Endocrine Disruptor Screening Program. Validation is the process by which the reliability and relevance of test methods are evaluated for the purpose of supporting a specific use (NIEHS, 1997). The contractor shall provide management, testing, and consulting services that will allow the Agency to develop, demonstrate, standardize and validate endocrine disruptor screens and tests.

The contractor shall design and perform toxicity test demonstrations and comparative studies, and summarize data and information necessary to standardize and validate a suite of endocrine disruptor screens and tests. The resultant data and information will be synthesized by the contractor into method specific technical reports. The method specific technical reports will be subjected to external peer review by the Agency using established procedures. The reports and peer review suggestions will subsequently be used by the Agency to promulgate test guidelines and associated technical guidance documents for use in Agency regulatory programs.

The general validation framework envisioned by EPA for endocrine disruptor screens and tests follows the general principles described by the Inter-agency Coordinating Committee for the Validation of Alternative Methods or ICCVAM (NIEHS,1997).

The endocrine disruptor screening battery contains *in vitro* and *in vivo* assays, whereas the

testing battery contains only *in vivo* assays. The *in vitro* assays and tests are not intended as animal replacement alternatives *per se*. However, the principles developed by ICCVAM are applicable to *in vivo* assays and necessitates consideration of reduced animal use, the refinement of procedures involving animals to reduce stress, and the replacement of animals in toxicological tests where feasible and practical. Although complete animal replacement is not currently feasible for many of the screens and tests (EDSTAC 1998, SAB/SAP 1999, NAS 1999) due consideration shall be given to reduced animal use and procedures to reduce stress in animals (NIEHS,1997).

3.2 Screens and Tests Requiring Standardization and Validation

The contractor shall manage the development and conduct of a variety of *in vitro* and *in vivo* biochemical, mammalian, and ecological toxicity test methods. During conduct of these tests, the contractor shall provide chemical analytical support concerning test article purity and stability, toxicity test dose preparation and verification, and analyses of the test article in complex media (feed, water, tissue, etc.). The specific screens and tests in EPA's proposed EDSP require varying degrees of development, standardization and validation for regulatory use and are listed below.

Tier 1 Screening Battery

Estrogen (ER) and Androgen receptor (AR) binding assays
ER and AR assays with transcriptional activation
Steroidogenesis assay with minced testes
3-day uterotrophic assay
5 to 7-day Hershberger assay
20-day pubertal female assay w/ thyroid
Frog metamorphosis assay
Fish reproductive screen

Tier 1 Screening Battery Alternatives

20-day pubertal male assay w/ thyroid
Placental aromatase assay
Mammalian *in utero* developmental screening assay

Tier 2 Testing Battery

Two generation mammalian reproductive toxicity study with endocrine endpoints
Two generation avian reproductive toxicity study with endocrine endpoints
Two generation fish reproductive study with endocrine endpoints
Partial fish life cycle fish reproductive study with endocrine endpoints
Two generation mysid shrimp reproductive study with endocrine endpoints
Amphibian life cycle reproductive toxicity study with endocrine endpoints
Amphibian partial life cycle reproductive toxicity study with endocrine endpoints

Research concerning endocrine disruptors is rapidly evolving, and assays are currently being developed which may offer distinct advantages over the specific assays and species listed above. As such, additional or substitute methods for endocrine disruptor screening and testing employing other endpoints or species may be included in this standardization and validation program (e.g., DNA or gene expression assays; vertebrate hormone endpoints other than estrogen, androgen, and thyroid agonists and antagonists; invertebrate hormones, etc.).

3.3 Management and Coordination of Standardization and Validation Activities

The contractor shall assist EPA in the management of the standardization and validation process. The contractor shall procure the services of laboratories for the standardization and validation program. The contractor is responsible for identifying the lead laboratory, selecting either themselves or another laboratory in consultation with the EPA Project Officer. The lead laboratory shall be responsible for the procurement and management of chemicals used in the standardization and validation program. This includes the preparation, analytical verification (purity, stability, etc.), and coding of samples, distribution of samples to participating laboratories, and storage of chemicals used as test articles. The lead laboratory shall also instruct participating laboratories on the use of the protocol and any novel or non-standard techniques and shall answer questions regarding protocol interpretation. The lead laboratory shall also collect, statistically

analyze and interpret data from inter-laboratory trials.

3.4 Expert Consultation and Special Studies

The contractor shall provide specialized expertise as needed in a consultant capacity or to perform experiments, both of which will aid in the selection of a screening method or help resolve issues related to protocol design or other technical problems encountered in the development and standardization of an assay. This may include toxicologists, ecotoxicologists, biochemists, statisticians, endocrinologists, analytical chemists, and biologists who have expertise with the assays or tests identified in the statement of work (SOW).

3.5 Pre-validation Studies

The contractor shall undertake prevalidation studies to develop, demonstrate and standardize those assays identified in the SOW. The contractor shall search and critically review the relevant toxicological literature to determine laboratory experience with the candidate screening and test methods to support the design of initial draft protocols. This analysis shall be presented in a Background Review Document (BRD) which will be used by EPA to establish the extent to which a candidate assay has been used and to delineate differences in protocols and practices. . The contractor's analysis shall provide the basis for drafting an initial protocol and determining what specific work needs to be done to resolve outstanding methodological issues. Where an assay has been conducted using different techniques or procedures from those identified in this SOW, the contractor shall prepare a table (or information in another appropriate format) showing the differences and questions that may require resolution through targeted pre-validation studies. Based on literature, experience and laboratory studies, the contractor in consultation with EPA shall develop protocols for use in pre-validation studies. The contractor may also

conduct laboratory studies to resolve methodological, procedural or technical issues.

The contractor shall conduct pre-validation studies to demonstrate the efficacy of a method, compare the sensitivity of the endpoints included in the protocol, obtain measures of intra-laboratory variability and optimize the protocol. The final product of pre-validation is an optimized transferrable protocol that can be used in a multi-laboratory validation study.

Pre-validation protocols and study designs shall be approved by EPA. Pre-validation studies shall be conducted in a single laboratory using a number of compounds selected by EPA. At least two trials shall be conducted using each draft protocol to determine repeatability in a single laboratory and the magnitude of intra-laboratory variability. The protocols used for the pre-validation studies shall be reviewed at the end of the pre-validation phase and modified as necessary to develop a standard transferable protocol for use in subsequent validation studies.

3.6 Validation Studies

The purpose of validation is to determine the ability of other laboratories to conduct the assays following the standard protocol and to establish the inter-laboratory variability and thus the reliability of the methods for use in the EDSP. In accordance with contract requirements, the contractor shall identify and select subcontractors to participate in the validation phase and submit its selections to the EPA Project Officer for approval.

The contractor shall prepare training materials to instruct the selected laboratories on how to conduct the validation studies. Validation study designs shall be approved by EPA. All laboratories conducting validation studies will strictly adhere to the EPA approved protocol and use test materials supplied by the lead laboratory. The contractor will prepare validation reports using data and information from the participating laboratories. Those reports will summarize,

synthesize and interpret the results from the individual laboratories as well as the interlaboratory variabilities.

3.7 Analysis and Reports

The contractor will evaluate the prevalidation and validation reports to determine if the findings, conclusions and recommendations are supported with defensible data.. The contractor will synthesize and interpret that data into method specific documents. Those documents will serve as the reference point for developing test guidelines to be used by the regulated community. They will also be submitted by EPA for independent scientific peer review. All primary data and measurements of laboratories participating in the validation program will be included in the peer review.

3.8 Quality Assurance/Quality Control

As required in the EPA Good Laboratory Practices (GLPs) Standards (40 CFR Part 792 and 40 CFR 160 Part 1) the contractor shall operate an independent quality assurance unit (QAU) to ensure that all studies are conducted under an appropriate Quality Assurance/Quality Control Program. For this procurement two levels of Quality Assurance/ Quality Control will be employed. All pre-validation studies will be conducted under a project specific Quality Assurance Program (QAP) developed by the contractor and approved by EPA. The QAPs for pre-validation studies do not need to comply with the full GLP. QAPs must include a quality assurance plan, QAU review of protocols, inspection of laboratories during the conduct of representative studies, and QAU review of reports to ensure compliance with protocols.

All validation studies will be conducted according to GLPs. The contractor will insure that its laboratories, those of its subcontractors, and those of other participants in the validation

studies are in compliance with the GLPs. In addition, EPA or its agent will conduct an independent laboratory/quality assurance audit of facilities participating in the validation program**.**

3.9 Preparation of Test Guidelines and Technical Guidance

The contractor shall prepare test guidelines and technical guidance to be used by test sponsors to comply with the regulatory testing requirements of the EDSP. The test guidelines will be developed using the standard protocols developed for the inter-laboratory validation program and will reflect the results of validation studies. Formats for those guidelines will be specified by the EPA Project Officer. The test guidelines will be peer reviewed by EPA and the FIFRA Scientific Advisory Panel and made available for public comment prior to approval and promulgation by the Agency.

3.10 Preparation of Standard Evaluation Procedures

Based on the results of the validation program, other information and in consultation with the EPA Project Officer, the contractor shall prepare guidance documents for the interpretation of data for the screens and tests selected by EPA for use in the Tier 1 screening battery and Tier 2 testing battery. The purpose of these Standard Evaluation Procedures is to ensure a general consistency in the interpretation of studies by EPA and contractor staff. The SEPs shall identify the major endpoints of each study and provide general guidance on how to interpret the results. EPA will provide formats for the SEPs and will also review and approve them.